AMERICAN SOCIETY FOR MICROBIOLOGY

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98TH GENERAL MEETING

MAY 17-21, 1998

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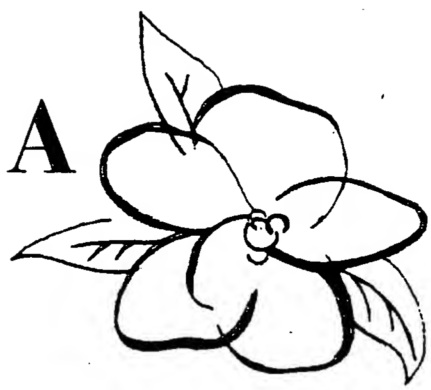

ABSTRACTS

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enty-six positives by all three methods and specimen combinations. Discrepant results were resolved by either DFA or another in-house PCR. EIA was able to detect 10.8% positive (81/752), PCR on urine alone detected 12.9% (97/752), while PCR on the urine mixed with cervix cells detected 13.6% (102/752). In the second part of the study, another two hundred and twelve pairs of urine and urine plus cervix cells were added to the study for the comparison of these two specimen types. The combined data yielded a positive rate of 12.3% (119/964) for urine alone and 13.1% (127/964) for urine plus cervix cells. PCR on urine alone or urine plus cervix cells are superior to EIA on cervix and urethral swabs combination. There is only slight advantage of adding cervix cells over urine alone when PCR is used for detection.

C-41. Use of Target Capture as a Sample Processing Method for the Simultaneous Detection and Differentiation of *C. trachomatis* and *N. gonorrhoeae* With Transcription Mediated Amplification (TMA)

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A sample processing method has been developed that selectively purifies target nucleic acid molecules away from both inhibitory substances and non-target nucleic acid. By the use of two capture oligomers, the rRNA of both Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) can be simultaneously extracted from a urine or swab sample. The capture oligomer contains a sequence homologous to a specific region in the target molecule as well as a string of deoxyadenosines. The capture oligomer:target complex can be captured on magnetic particles to which deoxythymidine oligomers have been covalently attached. The magnetic particles can be washed to remove impurities, and the samples can be added directly to a single TMA reaction. Both targets can be detected simultaneously by a dual kinetic hybridization protection assay. Because targets are captured directly from the specimen, intact cells are not required. Urine samples (n=18) mixed with a stabilizing transport buffer could detect the equivalent of a single GC or CT cell after 13 weeks of storage at 4°C or 25°C. When 10 GC or CT cells were added directly to urine samples (n=12) target could be detected after 72 hours at 25°C. Assay performance was not impacted when urine samples ranging from pH 5.0 to pH 8.0 were tested. Blood, a known inhibitor to amplified assays, was added to urine at 10% (v/v) final concentration with no inhibition detected. This assay format will be fully automated on the TIGRIS instrument.

C-42. Semi-Automation of the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay on the TECAN Genesis Liquid Handling System

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Gen-Probe's AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) is a very sensitive test for detecting *Chlamydia trachomatis* in either swab or urine specimens. Being a manual assay, AMP CT is especially suited for laboratories with a throughput requirement of up to 50 tests per day. High volume laboratories would benefit from automation, particularly for specimen processing. We applied the TECAN Genesis RSP 100 liquid handling system to the AMP CT assay for swab specimens. We used the TECAN Genesis to automate all of the pipetting steps for specimen preparation, two mixing steps, and pre-amplification reagent additions. Side-by-side comparison between manual and automated AMP CT using 92 clinical specimens in two batches showed perfect correlation. A series of additional experiments on the TECAN Genesis with over 600 samples demonstrated no evidence of cross-contamination and no front-to-back effects for batches as large as 182 tubes. Furthermore, one person can run over 500 tests per day using one TECAN Genesis RSP 100 instrument, making the application a suitable method for high-throughput chlamydia testing.

C-43. Comparative Evaluation of the PACE 2, AMP CT and LCx Assays for the Detection of *Chlamydia Trachomatis* in Endocervical and Male Urethral Specimens.

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We compared the AMPLIFIED Chlamydia Trachomatis (AMP CT) and PACE 2 assays (Gen-Probe, Incorporated), with the LCx assay (Abbott Laboratories), for the detection of *Chlamydia trachomatis* in endocervical and male urethral specimens. Paired samples from 354 patients were run in all three assays. Results for the three assays were reported on each patient to the responsible collection site. Patients were considered to be positive for *C. trachomatis* if samples were positive by two or more tests. One sample was positive by only the AMP CT test but was confirmed to be positive by Transcription-Mediated Amplification (TMA) testing using an alternate rRNA molecule. There were a total of 24 confirmed positive samples for a prevalence of 6.8%. Sensitivity was 83.3, 95.8 and 100% for the PACE 2, LCx and AMP CT assays respectively. All assays demonstrated 100% specificity. We conclude that the amplified assays demonstrate higher clinical sensitivity than the PACE 2 assay, but cost and workflow issues do not justify conversion from PACE 2 to amplified assays for routine testing in our laboratory. The PACE 2 assay will continue to be used for routine testing, while AMP CT testing will be offered only for female patients with recent exposure to a known or suspected positive contact, and for all male patients.

C-44. Choosing A Sensitive and Specific *Chlamydia Trachomatis* Assay Without Increasing Patient and Laboratory Costs

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Determining which *Chlamydia trachomatis* assay to use in a diagnostic setting can be an overwhelming task. Issues related to test sensitivity, specificity, patient costs and technical skill of the laboratory staff all influence the assay which is chosen. From January 1997 to August 1997 we evaluated the Gen-Probe® PACE® 2 and Amplified (TM) *Chlamydia trachomatis* assays to determine which would serve as the better replacement for our present EIA method. Test sensitivity, specificity, laboratory costs, patient charges and technical expertise were all considered. A total of 208 cervical samples were collected by trained Adolescent Medicine providers. Two swab samples were collected from each patient and randomized prior to collection to prevent bias. One sample was placed in chlamydia culture transport media and the other in Gen-Probe transport media. Both were transported to the lab. The culture samples were inoculated onto McCoy Cells and incubated 48-72 hours before harvest and immunofluorescence staining. The Gen-Probe® samples were processed and split to PACE® 2 and TMA aliquots upon arrival in the lab. These samples were frozen and tested as per manufacturers protocol at a later date. Of the samples which were cultured, 17 (8.2%) were positive, 182 (88%) were negative and 9 (4.3%) yielded inconclusive results. Samples tested by PACE® 2 and TMA yielded similar results with 19 (9.1%) testing positive and 189 (90.9%) testing negative. The sensitivity and specificity of both PACE® 2 and TMA compared to culture was 100% and 98.9% respectively. 4.3% of the culture data was essentially lost due to inconclusive results and made full analysis using culture as the gold standard difficult. In addition, TMA detected 2 positives which culture did not. To accommodate this, the TMA test was used as the gold standard, with discrepants being blindly tested by another lab. Using TMA as the gold standard, the PACE® 2 sensitivity was 94.7% and specificity was 99.5%. Compared to the sensitivity and specificity (80% and 97% respectively) of our current EIA method, a significant improvement in testing would be achieved using the PACE® 2 methodology. In addition, cost data obtained from the vendor indicated a lower patient charge would be generated using the PACE® 2 assay compared to culture, TMA and EIA. Since the PACE® 2 assay is performed in a manner similar to EIA, technical training to bring in this assay would be minimal. Given the increase in test sensitivity from our present EIA method, improved specificity, decreased patient charges and technical ease of the assay, it was decided that PACE® 2 will be used for routine *Chlamydia trachomatis* screening on cervical, urethral or conjunctival samples and to reserve TMA for testing urine samples.

C-45. Evaluation of Digene CT/GC Hybrid Capture II Test for the Identification of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Cervical Specimens

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The Digene CT/GC test is a nucleic acid probe-based chemiluminescent assay to detect DNA of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) in a single specimen. It uses a signal amplification method that couples hybridization to an antibody capture microplate system. The target DNA hybridizes with CT or GC RNA probes. The assay is performed in two steps. The initial test indicates the presence of either CT or GC in the sample. The second test identifies the specific organism. We evaluated the test with cervical specimens collected from 415 women seen at a San Francisco STD clinic. Results were compared to tissue culture (TC) isolation of CT in McCoy cells (using a blind pass and a DFA stain) and to GC culture (Thayer Martin and sugar utilization tests). Discrepant specimens (Digene+/culture-) were resolved by either a cytospin DFA stain or LCR test for CT and an alternate amplified test for GC. Compared to true positives, Digene CT had a sensitivity of 100% (21/21) and a specificity of 99.4% (392/394). TC sensitivity was 71.4% (15/21). Overall prevalence of chlamydia was 5.1%. Final culture results and discrepant analysis for GC are still pending. The Digene assay is a rapid convenient test that is an alternative to TC culture. With cervical specimens, the performance profile of Digene CT is better than culture. Further studies are needed to validate the assay with other types of specimens.

C-46. Evaluation of the Gen-Probe Amplified *Chlamydia Trachomatis* Assay (AMP CT) on Self-Collected Vaginal Swabs from Asymptomatic Young Females

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AMP CT is a transcription-mediated nucleic acid amplification assay that targets *C. trachomatis* ribosomal RNA. AMP CT on endocervical, vaginal (self- and clinician-collected), urethral and first catch urine (FCU) specimens was evaluated in an ongoing three-site study of asymptomatic women 16-25 years of age undergoing routine gynecological exams. AMP CT performance was compared to cervical and urethral tissue culture (TC) results. Apparent false positives (AMP CT+/TC-) were resolved by DFA and/or alternate amplification methods. Of 745 women who were screened, 63 (8.5%) had at least one chlamydia positive specimen. Compared to the infected patient as the gold standard (i.e., a confirmed positive specimen), AMP CT had the following sensitivities: 82.5% (cervical), 85.7% (urethral), 87.3% (vaginal-self), 82.5% (vaginal-clinician) and 65.6% (FCU). Specificities for all specimen types were

(1.6%)were initially COBAS negative and manual PCR positive with fewer than 1000 c/ml on interferon therapy and were COBAS positive on repeat. Two samples (1.6%) had repeated COBAS inhibition in internal control but tested positive by all other methods and by the second generation COBAS HCV assay. Overall, the COBAS Amplicor assay was a sensitive, specific and labor-saving method for detection of HCV RNA in patient sera.

C-132. Use of Target Capture in the Gen-Probe Quantitative HIV Assay to Remove Potential Interfering Substances

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The Gen-Probe HIV-1 Viral Load Assay is a rapid, simple and sensitive assay developed for the clinical lab to quantitate Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma from HIV-1 infected patients. A new specimen processing method is employed to purify target RNA from the specimen matrix. Transcription-Mediated Amplification (TMA) is used to amplify purified RNA target, followed by a homogeneous chemiluminescent detection assay (HPA).Purification of RNA from human plasma is accomplished through detergent lysis of viral particles followed by target capture of genomic viral RNA onto magnetic microparticles. Nucleases are inactivated during the lysis step and potentially interfering substances are removed through two wash steps. The potential to totally remove interfering substances with a reproducible sample processing method may obviate the need for an internal amplification control. In order to validate this assertion, we have tested a wide variety of plasma samples, including potentially interfering icteric and lipemic samples and patient samples containing non-target viruses. One hundred and fifty negative plasma samples were spiked with a constant level (1000-2000 copies per ml) of wild-type HIV virus and then quantitated with the Gen-Probe HIV-1 Viral Load Assay. In all plasma samples tested, the copy level predicted by the assay was within 0.3 log of the signal obtained with a control specimen. We have also demonstrated that the assay gives equivalent performance with ACD-, EDTA- and Heparin-stabilized plasma samples. In addition, testing of non-B subtype specimens (72 culture supernatants and 40 patient samples) demonstrated that the assay detects all known subtypes of HIV-1, including HIV-1 group O.The ease of use, sensitivity, precision and dynamic range of the Gen-Probe HIV-1 Viral Load Assay, combined with the potential of the sample processing method to eliminate interfering substances, make the assay a useful tool for monitoring a patient's HIV-1 disease progression or response to anti-retroviral therapy.

C-133. Sample Preparation for the Roche Amplicor HCV Monitor Assay

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The coefficient of variation for the Roche Amplicor HCV Monitor Assay can be as high as 40-50%. In an attempt to improve precision and efficiency, as well as reduce the risk of repetitive hand trauma, a procedure for resuspension of the final precipitated pellet was evaluated. This study compared resuspension by the Roche manual scraping method to bead resuspension using washed polystyrene Abbott Reagent Blanking Beads. Beads used in the study were washed twice in deionized water on an Abbott Parallel Processor and then twice in Roche HCV Specimen Diluent. The washed beads were stored in a sterile container until needed. Extraction of the patient plasma was performed in two mL microcentrifuge tubes. Kit controls (positive and negative) were extracted in a similar fashion. Following alcohol-precipitation of extracted samples, one mL of HCV Specimen Diluent was added to each tube. For each patient sample, pellets from three aliquots were resuspended manually while three were resuspended by vortexing for one minute in the presence of a bead. All aliquots of resuspended RNA were vortexed for five seconds prior to addition to the Amplicor Master Mix. Patient sets were amplified and analyzed within the same production run. The number of HCV RNA copies/mL was calculated for each aliquot and the means of the triplicate values were compared. Preliminary results demonstrated that RNA values were 5.9% to 36.9% higher following bead resuspension than for the manually extracted samples. Although variation among the triplicate values was somewhat greater following bead resuspension (mean CV = 12.7%) than for the manually extracted samples (mean CV = 10.4%), the difference was not significant. Bead resuspension created less hand/wrist trauma than the manual method. Additional studies are in progress to assess accuracy and precision of the bead resuspension method.

C-134. The Nuclisens™ Extractor: Use of Automated Nucleic Acid Isolation in Assessing HIV-1 Viral Load

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Accurate viral load measurements are necessary during monitoring HIV-1 infected patients under therapy. Apart from the nucleic acid quantitation method, correct viral load assessment depends on the specimen type, sample handling and the method of nucleic acid isolation. The nucleic acid isolation method of choice should: (1) obtain nucleic acid efficiently, (2) avoid accumulation of compounds interfering with subsequent amplification reactions and (3) exclude sample-to-sample contamination. The method described here is based on the binding of nucleic acid in the presence of chaotropic agents onto silica particles (also often referred to as the so-called Boom method). Purified material is obtained by washing the silica with several solvents and, after drying of the silica, elution of nucleic acid from the particles. The NucliSens™ Extractor has been designed to carry out the isolation of nucleic acid automatically with the aim to improve the method in view of vulnerability to contamination and labour intensity. Each sample mixture is loaded in a disposable cartridge containing a filter to achieve bound-free separation. The cartridges are connected to dedicated fluid displacement circuits for the washing steps and elution, enabling one to ten completely contained extractions within an hour. Performance studies of the Extractor revealed that HIV-1 RNA present in plasma (0.2 or 2.0 ml) and serum (0.2 ml) is recovered efficiently and that viral loads are identical to those obtained with the manual silica based isolation module of the NucliSens™ HIV-1 QT assay. In addition, all evaluated Extractors show very similar recoveries or viral loads. No significant interference with the NASBA reaction or any cross-contamination has been observed.

C-135. Evaluation and Usefulness of HCV RNA Quantitation Using Branched DNA, Monitor and NASBA in Patients with Chronic Hepatitis C Treated with Interferon

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Pretreatment viral load and response to interferon (IFN) are closely associated and the quantitation of HCV RNA before and during treatment may be important. We have evaluated 3 quantitative (QT) assays for HCVRNA quantitation: bDNA 2.0 (Chiron), Monitor (Roche) and NASBA (Organon Teknika), in comparison with a RT-PCR. The cutt-off values of the assays were respectively 2.105, 103 and 3.103 copies/ml. We studied the Eurohep panel with genotype 1 (G1) and 3 (G3) standards, and samples before IFN, after 3 (M3) and 6 (M6) months of treatment from 35 patients (pts) with chronic hepatitis C, treated with 3 to 6 MU of IFN during 6 months. At M3 and M6, pts with normal ALT were considered as biochemical responders (BR), pts with HCVRNA negative as virological responders (VR) and pts with abnormal ALT as non responders (NR). Results: bDNA failed to quantify the Eurohep panel as expected. For G1: RT-PCR gave + results until the 1/4000 dilution and Monitor and NASBA until the 1/1000 dilution. For G3: RT-PCR gave + results until the 1/1000 dilution and Monitor and NASBA until the 1/10 dilution. Before IFN, all the 35 pts had detectable HCVRNA by PCR, NASBA and Monitor. bDNA failed to detect 5 samples. At M3, 22 pts had a BR. 16 pts samples were - using RT-PCR and the 3 QT assays and 6 were + using PCR and NASBA, but undetectable through bDNA or Monitor. 13 pts were NR. At M6, 22 and 13 pts were also BR or NR. 12/16 pts had - HCVRNA using RT-PCR and the 3 QT assays at the end of IFN (M6) and were still - 6 months after the end of IFN. bDNA failed to detect 2 samples and Monitor one. Another RT-PCR + sample was missed by the 3 QT assays. We found a 1log10 difference in the level of viremia between the 3 tests and quantitation of genotype 2, 3 and 4 seems underestimated by Monitor. In conclusion, the sensitivity of the NASBA QT seems very close to RT-PCR and may be useful in monitoring antiviral treatments. Standardization between quantitative HCVRNA assays is till required.

C-136. Rapid Detection of HCV PCR Products by a DNA Enzyme Immunoassay

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PCR remains the method of choice to diagnose chronic hepatitis C virus infection. Most laboratories use an "in house" developed PCR assay that amplifies a unique sequence from 5' non coding region of the viral RNA. Generally, PCR products are detected with agarose gel electrophoresis followed by Southern blot hybridization. DNA Enzyme Immunoassay (DEI) is based on the hybridization of amplified DNA to a biotin labeled capture probe that is linked to streptavidin coated ELISA wells. The hybrid between probe and DNA is then detected with antibody to double stranded DNA. To evaluate DEI for detecting HCV PCR products, serial 10 fold dilution of HCV amplicons were detected by both DEI and agarose gel elcetrophoresis followed by Southern blot hybridization with a 3'end labeled chemiluminescent probe. Results from these studies showed that DEI was as sensitive as Southern blot hybridization for detecting HCV PCR amplicons. Similarly, PCR was performed, prospectively, on 100 sera from patients with suspected chronic HCV infection. There were 36 specimens positive for HCV by PCR that were detected by both methods (100% concordance). Agarose gel electrophoresis alone detected 30 (83%) of the positive samples. While, agarose gel electrophoresis followed by Southern blot hybridization detected all the positive samples, it required over night blotting and several hours of hybridization and detection to attain a final result. Incontrast, the results of DEI were available within 3 hours of amplification. In addition, DEI was not PCR specific which enabled simultaneous detection of multiple PCR products generated from variety of sources.

C-137. MedMira Rapid HIV Screen Test: A Multinational Field Trial

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MedMira Rapid HIV Screen is a newly developed immunodot test for rapid detection of HIV-1/2 antibodies. A multinational study was done using repository and fresh serum/plasma specimens to determine the performance characteristics of this test in different geographic settings. In Canadian study, based on 1,147 sera tested (210 HIV-1 +ve and 937 HIV-ve), the sensitivity was 99.5% and specificity 99.6%. In Ugandan study, based on 829 sera tested (461 HIV +ve and 368 HIV -ve), the sensitivity was 100% and specificity 98.6%. In Sierra Leone study, based on 300 sera tested (100